

SPECIFIC DISINTEGRATION OF NEMATOCYSTS IN *HYDRA JAPONICA* BY TREATMENT WITH PROPARGYLGLYCINE

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ABSTRACT

When *Hydra japonica* was treated with 2 mM D,L-c-propargylglycine, a specific inhibitor of γ -cystathionase, for 75 h, the number of desmonemes present in the tentacles dropped drastically. H. isorhiza and A. isorhiza numbers also showed smaller drops, but the stenotele number remained unchanged. Total cell numbers and cell composition except for the battery cells and nematocytes, remained unchanged. The disappearance of the mature desmonemes was apparently due to their specific disintegration in the living hydra, and not to inhibition of differentiation from interstitial cells to nematocytes, and/or their migration, nor accelerated nematocyst discharge from the battery cells. Hydra lacking desmonemes may be useful in investigating how the proportions of each nematocyte type are kept constant, and how nematocyte differentiation is regulated.

INTRODUCTION

Hydras have many nematocytes, which are classified into four types: stenotele, desmoneme, atrichous isorhiza (A. isorhiza), and holotricous isorhiza (H. isorhiza), according to their structure and functions (Mariscal, 1974). They differentiate from the interstitial cells in the intercellular space of the body ectoderm (David and Challoner, 1974). Most mature nematocytes, especially desmonemes and A. isorhizas, migrate to the tentacles and are retained in the battery cells (Campbell, 1967; David and Gierer, 1974). The ratio of nematocytes to interstitial cells and the proportions of each nematocyte type are kept constant throughout asexual growth (Bode *et al.*, 1977). The mechanism for keeping the stem-cell pool constant has been investigated (Bode and David, 1978), but the mechanism which keeps the proportions of each nematocyte type constant is unknown. Hydras that lack a particular type of nematocyte may be useful in investigating this latter mechanism.

In studying nematocyte differentiation, we found that the number of nematocyst capsules in the tentacles decreased drastically when hydras were treated with D,L-c-propargylglycine (PG), an inhibitor of γ -cystathionase, the enzyme for L-cysteine biosynthesis (Washien and Abeles, 1977). The effects of PG on hydras, especially on nematocysts, were then examined in detail. We found that desmonemes disappeared because they disintegrated. This treatment provides a method of preparing large numbers of hydras lacking desmonemes and A. isorhizas.

MATERIALS AND METHODS

Culture of hydra and D,L-c-propargylglycine treatment

The *Hydra japonica* used for all experiments were mass cultured in a solution containing 1.0 mM NaHCO₃, 0.3 mM CaCl₂, 0.06 mM KCl, 0.06 mM MgCl₂,

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Abbreviations: PG: D,L-c-propargylglycine; SDS: Sodium dodecyl sulphate; H. isorhiza: Holotrichous isorhiza; A. isorhiza: Atrichous isorhiza.

and 0.1 mM EDTA·4Na (pH 7.8–8.0) in distilled and deionized water at $20(\pm 1)^{\circ}\text{C}$ according to the methods of Hirakawa and Kijima (1980). Large hydras without buds, which had been starved for 2 days, were exposed to 2 mM D,L-propargylglycine (PG) in the culture medium for various periods. The medium was changed daily and animals were not fed during the experimental period.

Counting of nematocysts and cell type

Twenty hydras were transferred to a tube and rinsed with about 5 ml culture medium. A nematocyst suspension was prepared by dissolving the hydras in 0.5 ml or 1.0 ml culture medium containing 2% sodium dodecyl sulphate (SDS). Total nematocysts and the number of each kind in the suspension, then, were counted with a blood-corpuscle counting chamber under a phase contrast microscope at $\times 400$ (Bode and Flick, 1976). The error bars in the figures represent the standard errors of means in three to five experiments.

Hydra was macerated to quantify each cell type. Maceration and cell identification followed the procedure of David (1973).

RESULTS

Effects of PG on morphology and behavior

The tentacles of animals exposed to PG became shorter and smoother than untreated tentacles. Figure 1 shows part of a hydra tentacle treated with 2 mM PG for 72 h and a control tentacle. Battery cells in the tentacles of a control hydra contained many nematocytes with one cnidocil on the apex of each nematocyte. One stenotele at the center of the battery cell was surrounded by about 15 desmonemes. A few *H. isorhizas* and *A. isorhizas* also were often in the battery cell (Fig. 1a). In the tentacles of treated hydras (Fig. 1b), most of the desmonemes and their cnidocils disappeared, but stenoteles remained unchanged. The nematocyst capsules remaining in the tentacles seemed a little smaller, and the thread forming a corkscrew like coil in the desmoneme's capsule was also not visible.

The behavior of treated hydra (such as spontaneous column and tentacle contractions) was almost normal except for the feeding behavior and foot attachment. The feeding behavior, which is induced by prey or with glutathione, almost completely stopped. Normally, after feeding hydras adhere to the bottom, while starved hydras make a bubble at the foot, and float on the surface of the culture medium under the culture conditions employed. The PG-treated hydra, however, became adhesive to the bottom.

Effect of PG on number of nematocytes

A single untreated *H. japonica* had about 32,000 nematocytes, of which 76% were desmonemes, 10% stenoteles, 7.5% *H. isorhizas*, and 6.5% *A. isorhizas*. Each type of nematocyst was counted at various times during the treatment of hydra with 2 mM PG (Table I and Fig. 2). The number of each type barely decreased within the first 25 h of PG-treatment. Subsequently, the number of desmonemes decreased sharply to 20% of the control value at 75 h after the application of 2 mM PG (Fig. 2). *A. isorhizas* also decreased a little more slowly than desmonemes. *H. isorhiza* number decreased still more slowly, to 52% of control number in 50 h. On the other hand, the stenoteles did not decrease significantly during the 75

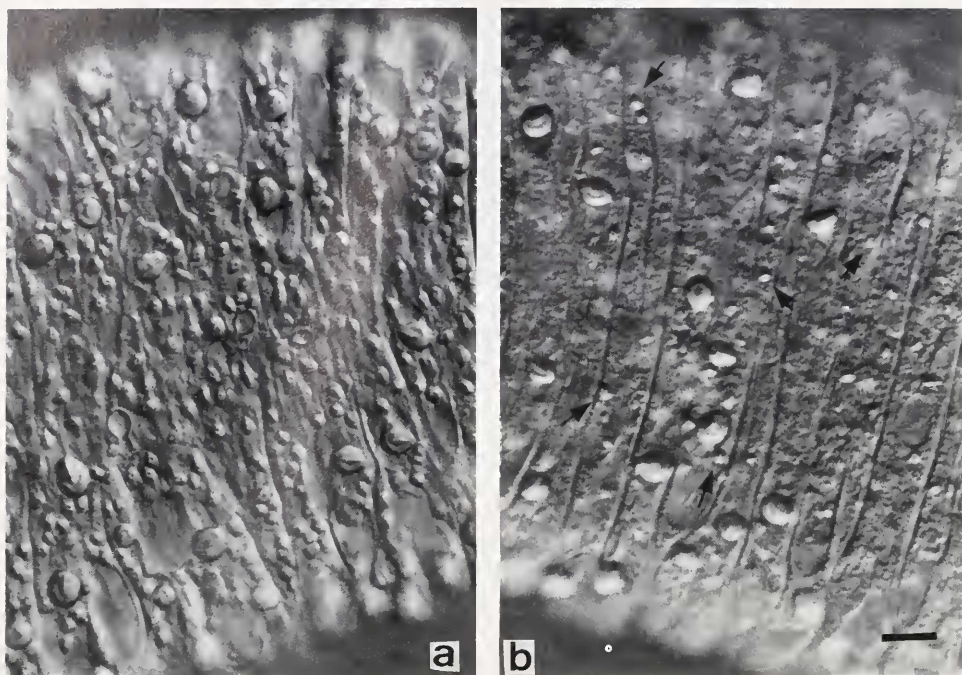


FIGURE 1. Photomicrographs of hydra tentacle observed under Normarski differential interference optics. a) control. b) 2 mM treatment for 75 h. Arrows indicate the few remaining and deformed desmonemes after treatment. Scale bar equals 20 μ m.

h treatment, but after the 150 h treatment stenoteles decreased to 43% of control level (Table I).

About 80% of all nematocysts were in the tentacles. This included 90% of desmonemes and 80% of *A. isorhizas*. About 70% of the stenoteles and 55% of the *H. isorhizas*, in contrast, were in the body. To examine whether the decrease in nematocyst numbers occurred mainly in the tentacles, hydras were cut into two pieces just below the hypostome region, and the numbers of nematocysts in each portion counted. In the tentacles, the numbers of desmonemes, *A. isorhizas*, and *H. isorhizas* dropped to about 10%, 25% and 30% of control level, respectively, after treatment with 2 mM PG for 75 h. The number of stenoteles did not decrease. In the body, the numbers of each kind of nematocyst were almost the same as in the controls. This showed that, except for the stenoteles, PG treatment decreased the numbers of nematocysts in the tentacles, but did not affect nematocysts in the body during the 75 h treatment.

Effect of PG on the cell population

The total cell number, excluding the nematocytes, in a typical hydra was 7×10^4 : about 40% epithelial cells, 37% interstitial cells, 7% nerve cells, and 16% other cell types (gland cells, mucous cells and nematoblast cells). PG did not change the total cell number except for nematocytes in the battery cells during 100 h

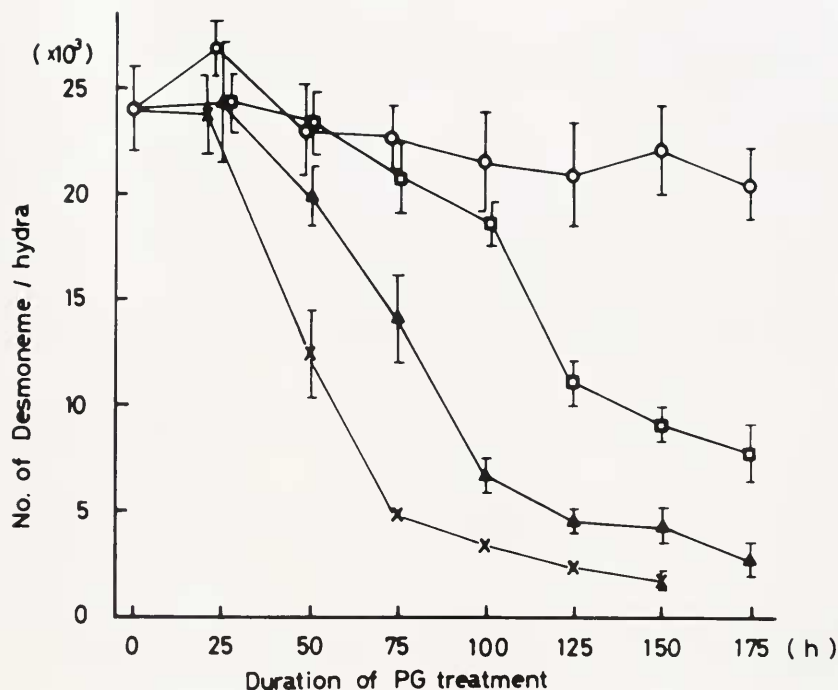


FIGURE 2. Time course of PG effects on desmonemes in a whole hydra. (O, control; □, 0.5 mM; Δ, 1.0 mM; X, 2.0 mM)

treatment. The proportions of each cell type were also little different from those in the untreated hydras.

Effect of PG on hydras in which the supply of desmonemes was blocked by cutting

In normal hydra, the number of nematocytes is kept nearly constant by balanced production and depletion (Bode and Flick, 1976). The decrease of the desmonemes in PG-treated hydra could be caused by inhibition of production, or acceleration of depletion, or both. Nematoblast cells differentiating to desmonemes are located in the gastric region, and absent just under the hypostome and in the tentacles (David and Challoner, 1974). Thus, the supply of newly matured desmonemes to the tentacles can be stopped for at least 60 h by removing the hydra's body just under the hypostome. The interstitial cells remaining in the hypostome might start to differentiate into desmonemes just after cutting, but it should take more than 60 h for differentiation from the final cell division to the matured nematocyte (David and Gierer, 1974).

Hydras were divided into two groups, I and II. Group I consisted of intact hydra while group II consisted of tentacles and hypostome only. Half of the animals of each group were incubated in PG (these were designated as PG-I and PG-II). The tentacles of group II animals (Control-II and PG-II) were not supplied with new desmonemes, while those of group I (Control-I and PG-I) were supplied during

TABLE I

The effect of 2 mM PG on the numbers of nematocysts in a hydra.

Duration of PG treatment		Desmoneme	Stenotele	Holo-trichous Isorhiza	Atrichous Isorhiza	Total Nematocyst
0 h	C	24,000 ± 2,000	3,050 ± 300	2,400 ± 450	2,000 ± 200	31,500 ± 2,650
25 h	C	26,900 ± 1,200	3,900 ± 450	2,450 ± 300	2,200 ± 400	35,500 ± 3,600
	PG	23,800 ± 1,800	3,150 ± 200	2,450 ± 400	1,800 ± 250	31,200 ± 2,300
	%	88.5	80.8	100	81.3	88.0
50 h	C	24,700 ± 2,100	3,800 ± 450	2,400 ± 300	2,000 ± 350	32,900 ± 3,000
	PG	12,450 ± 2,050	4,450 ± 500	2,200 ± 400	1,050 ± 250	20,150 ± 2,400
	%	50.5*	118.7	91.7	52.5*	61.2*
75 h	C	28,800 ± 4,600	3,900 ± 600	2,500 ± 400	2,050 ± 300	37,400 ± 5,750
	PG	4,850 ± 950	3,800 ± 400	1,300 ± 100	600 ± 150	10,600 ± 750
	%	16.8*	97.4	52.0	29.3*	28.3*
150 h	C	23,700 ± 2,100	4,650 ± 550	2,500 ± 400	1,700 ± 200	32,600 ± 2,900
	PG	1,700 ± 900	2,000 ± 200	500 ± 100	350 ± 100	4,550 ± 550
	%	7.2*	43.0*	20.0*	20.6*	14.0*

Data are the cumulative results of 5 or 6 independent experiments and are presented as mean ± SEM.

* Significantly different from untreated hydra at the 95% confidence level using *t*-test.

Abbreviations: C: untreated with PG, PG: treated with PG, %: per cent of PG to C.

the experimental period. If PG only inhibits the production of desmonemes and/or their migration to the tentacles, the number of desmonemes of PG-II should be the same as that of Control-II. On the other hand, if the decrease is due to depletion from the tentacles only, the rate of decrease in PG-II should be the same as that in PG-I. The numbers of desmonemes in the tentacles and hypostome in each of the four groups were counted after the intervals shown in Figure 3. For 30 h of treatment, from 20 h to 50 h, Control-I and Control-II remained almost unchanged. The numbers of desmonemes in PG-I and PG-II, however, showed a similar and drastic decrease. Thus, the rapid decrease of desmonemes was mainly due to acceleration of depletion rather than the inhibition of production or migration.

Effect of PG on the discharge of desmonemes

Two possible mechanisms for the depletion of desmonemes in the tentacles are (1) discharge of desmonemes into the external medium, and (2) their disintegration in the living tentacles. The mature nematocyst-capsule was so stable in the culture medium that all types of nematocysts remained intact for at least 3 days. Therefore, the discarded nematocysts could be estimated by counting nematocysts in the culture vessels. However, direct counting of discharged nematocysts was difficult, since their number was very small and nematocyst threads got tangled together when discharged nematocysts were enriched by centrifuging. So, the total number of nematocysts (both discarded and retained in the hydra) was counted and compared with the number of retained nematocysts only at various times during PG treatment. Figure 4 shows that the sum of the discarded and retained desmonemes was almost the same as the number of retained desmonemes in both control and PG treated

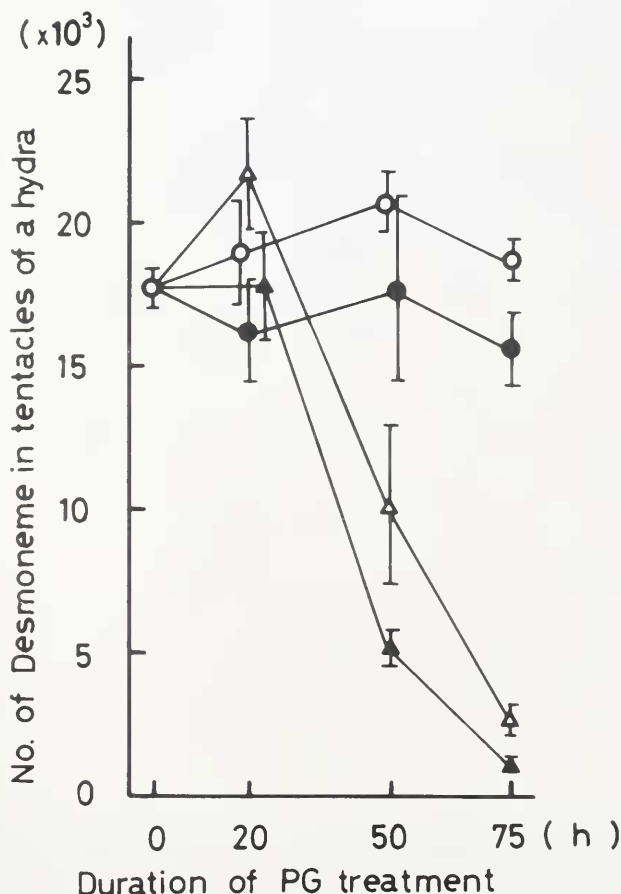


FIGURE 3. Effect of 2 mM PG on the desmonemes of hydra tentacles and hypostome with blocked supply of new desmonemes. Hydrazes in Control-I (O) and PG-I (Δ) were cut just under the hypostome immediately before counting, while animals in Control-II (\bullet) and PG-II (\blacktriangle) were cut just before the PG-treatment (at time 0), to block the supply of new nematocysts. Control-I and -II were not treated with PG.

hydrazes. This shows that the number of discarded desmonemes is negligibly small. The sum of retained and discarded desmonemes in treated hydrazes decreased markedly compared to controls. Thus, the decrease in desmonemes was not due to their discharge, but due to disintegration in living hydrazes.

Hydra with a few drops of 2 mM PG-solution were placed on a glass slide, and nematocysts were discharged by touching the hydra with a glass rod. The discharged nematocysts were observed under the microscope for 3 days. The nematocysts remained unchanged and did not disintegrate by direct action of PG. Thus, PG may act on a mechanism which maintains mature desmonemes in the living hydrazes, and the desmonemes may be degraded by metabolic action of living cells. This is supported by the fact that many small objects thought to be degrading capsules were observed in the living tentacles of PG-treated hydra (Fig. 1b) and in the solution where PG-treated hydrazes were dissolved by 2% SDS (Fig. 5b).

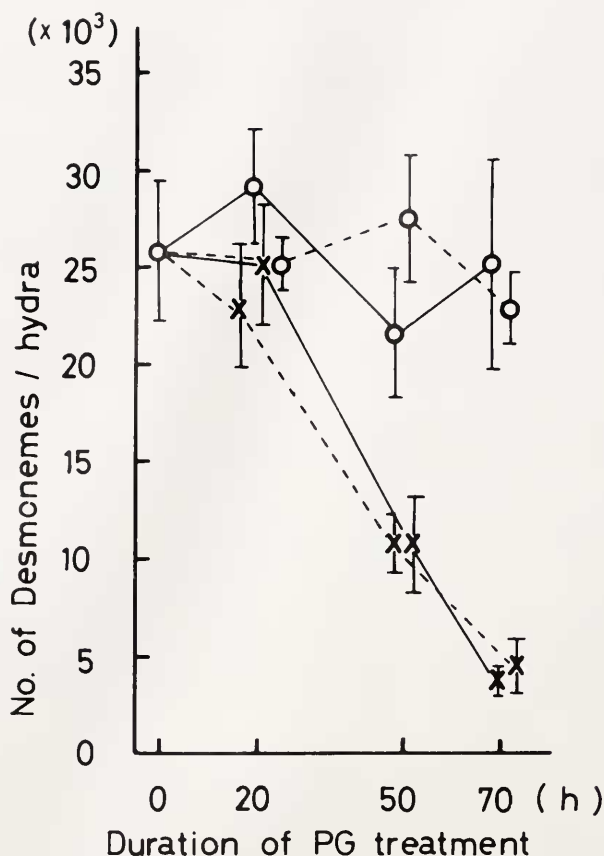


FIGURE 4. Effect of 2 mM PG on the discharge of desmonemes. Hydras were treated with 2 mM PG. At 0 h, 20 h, and 50 h of the treatment, twenty hydras were transferred to a separate tube, rinsed with about 5 ml test medium and were cultured in 0.4 ml test medium for 20, 30 or 20 hrs, respectively. 0.1 ml of 10% SDS was then added and the numbers of desmonemes counted (times 20 h, 50 h, and 70 h, respectively). These values represented the sum of both discarded and retained desmonemes (---○---, ---×---). The number of retained desmonemes only (—○—, —×—) was counted at 0 h, 20 h, 50 h, and 70 h by transferring twenty hydra to a separate tube, rinsing and immediately adding 10% SDS. Open circles represent numbers of desmonemes in control animals and crosses represent those treated with 2 mM.

Reversibility of PG effect

When hydras were transferred back to normal culture medium after 3 days of treatment, the decrease of nematocysts stopped. If transferred hydras were forced, increased nematocyte numbers and budding occurred. Nematocyst numbers returned to control levels in a week indicating that budding and nematocyte synthesis ability were not destroyed permanently by the PG treatment. The details of the recovery process will be reported elsewhere.

DISCUSSION

The experiments presented show that PG treatment drastically decreased the number of nematocysts, especially desmonemes, mainly due to the disintegration

of the nematocysts in living tentacles. PG's action differed depending on the type of nematocysts and their location. Stenoteles showed the least response to PG. All nematocyst types in the body column were not affected. Some nematocysts, including most desmonemes and *A. isorhizas*, migrate and do not remain in the body column. These results suggest that PG acts after the nematocyte migration.

The following results were obtained concerning the mechanism of nematocyte disintegration. (1) The capsules of remaining nematocysts became slightly smaller and were deformed (Fig. 1). The deformed nematocysts were often found in SDS suspension (Fig. 5). (2) The cnidocils of nematocytes on the tentacle disappeared after PG treatment. Sections of treated hydra showed few nematocyte nuclei under phase contrast microscopy. Thus, the capsule and the nematocyte may disintegrate almost simultaneously after a little shrinkage of the nematocyst capsule.

Campbell (1976) reported that colchicine treatment depleted the interstitial cells, nematoblasts, nematocytes, and nerve cells in *H. attenuata*, and that this depletion was due to phagocytosis by the digestive cells in the body. However, such phagocytosis probably is not responsible for the PG depletion of desmonemes because PG had little effect on desmonemes in the body region. Desmonemes were degraded in the tentacle where few digestive cells are located.

Mature nematocytes are discarded into the external solution after nematocyst discharge, or otherwise, nematocytes migrate to the tentacle tip and are discarded from the tip. Presumably, they never disintegrate inside normal living hydra. Even in a mutant hydra which synthesized stenoteles that did not migrate into the tentacles (Fujisawa and Sugiyama, 1978), stenoteles which accumulated in the body, presumably, did not disintegrate but were discarded into the gastroderm. However, PG treatment caused disintegration of mature nematocyst in living hydra.

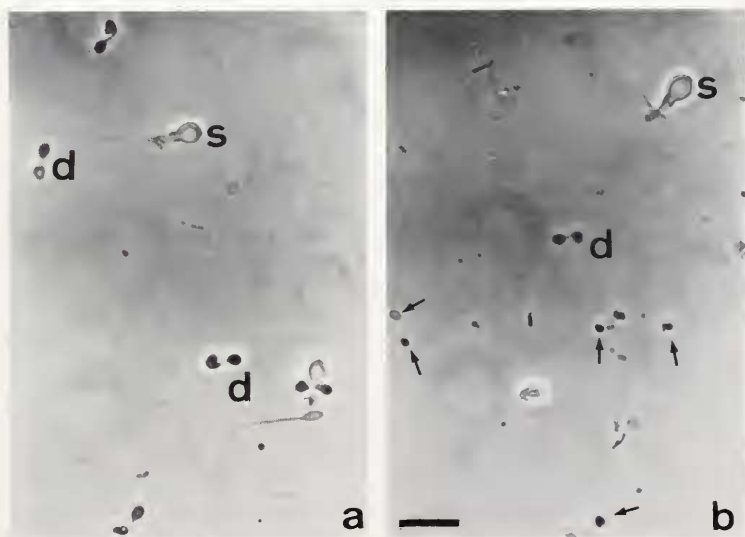


FIGURE 5. Photomicrographs under a phase contrast optics. The nematocyst capsules were obtained by dissolving hydras in 2% SDS solution. a) Control nematocysts. b) Those obtained from hydras treated by 2 mM PG for 75 h. In (b), many small objects indicated with arrows are, presumably, desmoneme capsules degrading (s, stenotele; d, desmonemes). Scale bar equals 20 μ m.

PG is a specific irreversible inhibitor of several enzymes, including γ -cystathionase (Washtien and Abeles, 1977) and aspartate aminotransferase (Tanase and Morino, 1976). Among these enzymes, γ -cystathionase, which catalyzes the final step of L-cysteine biosynthesis, is most strongly inhibited by irreversible binding of PG at the active site. We examined the antagonistic effect of L-cysteine. At low concentration (below 5 mM), L-cysteine partly repressed the effect of 2 mM PG on hydra. At higher concentrations (above 5 mM), it was toxic to the hydras. Therefore, its antagonistic effects could not be examined.

Three methods have been used to obtain hydras lacking some types of nematocytes. The first is chemical treatment, for example, with colchicine (Campbell, 1976) or hydroxyurea (Bode *et al.*, 1976). These treatments, however, also deplete the interstitial cells and nerve cells. The second is the genetic method of isolating mutants by inbreeding. Sugiyama and Fujisawa (1977) have obtained many mutant hydras, but no hydra which lack nematocytes only. Such a strain would be difficult to culture and propagate, since such hydras could not capture prey. However, one strain which possesses no stenoteles on the tentacles (Fujisawa and Sugiyama, 1978) has been isolated. The third method, electrical stimulation, causes discharge of most of the stenoteles, but not most other nematocysts, especially desmonemes (Zumstein, 1973).

Compared with the above three methods, PG treatment is an easy way to obtain many hydras with normal interstitial and nerve cells, but lacking nematocytes, especially desmonemes and A. isorhizas.

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LITERATURE CITED

- BODE, H. R., AND C. N. DAVID. 1978. Regulation of a multipotent stem cell, the interstitial cell of hydra. *Prog. Biophys. Molec. Biol.* **33**: 189-206.
- BODE, H. R., AND K. M. FLICK. 1976. Distribution and dynamic of nematocyte population in *Hydra attenuata*. *J. Cell. Sci.* **21**: 15-34.
- BODE, H. R., K. M. FLICK, AND P. M. BODE. 1977. Constraints on the relative sizes of the cell population in *Hydra attenuata*. *J. Cell. Sci.* **24**: 31-50.
- BODE, H. R., K. M. FLICK, AND G. S. SMITH. 1976. Regulation of interstitial cell differentiation in *Hydra attenuata*. I) Homeostatic control of interstitial cell population size. *J. Cell Sci.* **20**: 29-46.
- CAMPBELL, R. D. 1967. Tissue dynamic of steady state growth in *Hydra littoralis*. III) Behavior of specific cell type during tissue movement. *J. Exp. Zool.* **164**: 379-392.
- CAMPBELL, R. D. 1976. Elimination of hydra interstitial cell and nerve cells by means of colchicine. *J. Cell Sci.* **21**: 1-13.
- DAVID, C. N. 1973. A quantitative method for maceration of hydra tissue. *Wilhelm Roux' Archiv.* **171**: 259-268.
- DAVID, C. N., AND D. CHALLONER. 1974. Distribution of interstitial cells and differentiating nematocytes in nests in *Hydra attenuata*. *Amer. Zool.* **14**: 537-542.
- DAVID, C. N., AND A. GIERER. 1974. Cell cycle kinetics and development of *Hydra attenuata*. III) Nerve and nematocyte differentiation. *J. Cell Sci.* **16**: 359-372.

- FUJISAWA, T., AND T. SUGIYAMA. 1978. Genetic analysis of developmental mechanism in *Hydra*. IV) Characterization of a nematocyst deficient strain. *J. Cell Sci.* **30**: 175-185.
- HIRAKAWA, Y., AND H. KIJIMA. 1980. Behavioral analysis of glutathione receptor of *Hydra*. *J. Comp. Physiol. A.* **135**: 73-81.
- MARISCAL, R. N. 1974. Nematocysts. In: *Coelenterate Biology*. Muscatine, L. and H. M. Lenhoff, Eds. Academic Press: New York, Pp. 129-178.
- SUGIYAMA, T., AND T. FUJISAWA. 1977. Genetic analysis of developmental mechanism in *Hydra*. I) Sexual reproduction of *Hydra magnipapillata* and isolation of mutant. *Develop. Growth Differ.* **19**: 187-200.
- TANASE, S., AND Y. MORINO. 1976. Irreversible inactivation of aspartate aminotransferases during transamination with L-propargylglycine. *Biochem. Biophys. Res. Commun.* **64**: 1301-1308.
- WASHTIEN, W., AND R. H. ABELES. 1977. Mechanism of inactivation of γ -cystathionase by the acetylenic substrates analogue propargylglycine. *Biochemistry* **16**: 2485-2491.
- ZUMSTEIN, Z. 1973. Regulation der Nematocyten—Produktion bei *Hydra attenuata* Pall. *Wilhelm Roux' Archiv.* **173**: 249-318.